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Evaluation of serum insulin-like growth factor-1 and 26S proteasome concentrations in healthy dogs and dogs with chronic diseases depending on body condition score

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ABSTRACT

In patients suffering from chronic diseases, the objective assessment of metabolic states could be of interest for disease prognosis and therapeutic options. Therefore, the aim of this study was to assess insulin-like growth factor-1 (IGF-1) and 26S proteasome (26SP) in healthy dogs and dogs suffering from chronic diseases depending on their body condition score (BCS) and to examine their potential for objective assessment of anabolic and catabolic states. Serum concentrations of IGF-1, an anabolic hormone, and 26SP, a multiprotein complex which is part of the ubiquitin-proteasome pathway, by which the majority of endogenous proteins including the muscle proteins are degraded, were measured in 21 healthy dogs and 20 dogs with chronic diseases by canine ELISA. The concentrations of IGF-1, 26SP and their ratio (IGF-1/26SP) were set in relationship to the BCS of the dogs. When examining healthy and chronically diseased dogs separately, a positive correlation between IGF-1 and the BCS was observed in the healthy group and a negative correlation between 26SP and the BCS was noted in dogs with chronic diseases. Further, dogs suffering from chronic diseases showed higher 26SP concentrations and lower values for IGF-1/26SP than the healthy dogs. Overall, we detected a negative correlation between 26SP and the BCS and a positive correlation between IGF-1/26SP and the BCS. The results of our study indicate usability of IGF-1 for description of anabolic states, while 26SP could be useful for detection and description of catabolic states. Finally, the ratio IGF-1/26SP seems to be promising for assessment of metabolic states.

1. Introduction

In humans, chronic diseases like diabetes, chronic kidney disease, chronic heart failure and acquired immune deficiency syndrome (AIDS) are accompanied by muscle wasting and weight loss (Anker et al., 1997b; Lecker et al., 2004; Mitch and Goldberg, 1996). Additionally, skeletal muscle atrophy as a consequence of catabolic condition correlates with higher incidence of complications, worsening of the prognosis and declining quality of life (Anker et al., 1997b; Bergström, 1995; Carrero et al., 2008; Hülsmann et al., 2004; Strasser et al., 2007; Windsor and Hill, 1988). Thus, the objectification of anabolic and catabolic states could be of interest for diagnostic and therapeutic options and for disease prognosis. Until today, no blood parameters to objectively measure the metabolic state of dogs exist. Therefore, reliable biomarkers for the assessment of metabolic state in dogs are needed. Because anabolic states are induced by anabolic hormones and catabolic states are associated with protein degradation, we investigated the anabolic hormone Insulin-like-growth-factor-1 (IGF-1) and 26S proteasome (26SP) as part of the protein degradation system in this study.

IGF-1 is a 70-amino-acid polypeptide hormone (Delafontaine et al., 1993; Rinderknecht and Humbel, 1978), which is synthesised mainly in the liver, but also in a lot of other tissues, and is acting via the IGF-1-receptor (IGF-1R), the insulin-receptor (IR) and hybrid receptors (IGF-1/IR). IGF-1 is involved in the regulation of growth and metabolism and plays a critical role in cell cycle control (Le Roith et al., 2001). An important role of IGF-1 can be found in the regulation of the skeletal muscle metabolism. Because of the anabolic effect of IGF-1 on the skeletal musculature, protein biosynthesis increases and proliferation and differentiation of myoblasts and satellite cells in skeletal muscles are stimulated (Florini et al., 1996). Additionally, IGF-1 inhibits muscle wasting by constraining muscle cell apoptosis (Lawlor and Rotwein, 2000), proteolysis and the ubiquitin-proteasome-pathway (Chrysis and Underwood, 1999; Hong and Forsberg, 1994). IGF-1 is also of major significance for growth and differentiation of adipose tissue, stimulating both differentiation of mature adipocytes from adipocyte

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precursor cells and the growth of mature adipocytes (Blüher et al., 2005). Furthermore, in mature adipocytes IGF-1 increases glucose transport and lipogenesis and decreases lipolysis (Wabitsch et al., 1995).

26S proteasome is a large multiprotein complex. It consists of the central, cylindrical and proteolytically-active 20S proteasome and two regulatory 19S complexes (Coux et al., 1996). The 26S proteasome is part of the ubiquitin-proteasome pathway, which is the major pathway for selective protein degradation in eukaryotic cells. The majority of intracellular proteins are degraded by this pathway (Rock et al., 1994) and it is the most important proteolytic pathway during accelerated degradation of muscle proteins in catabolic conditions (Mitch and Goldberg, 1996). In recent years, detailed knowledge on the ubiquitin-proteasome pathway and protein degradation in the course of chronic diseases has been gained in human medicine. In addition to many other factors (e.g., glucocorticoids, cytokines, acidosis or inactivity), IGF-1 is involved in the complex signal transduction cascades regulating this pathway (Attaix et al., 2005; Lecker et al., 2006; Wang and Mitch, 2013).

Consequently, the aim of our study was to evaluate the potential of IGF-1 and 26SP as indicators of anabolic and catabolic states in dogs. Therefore, the IGF-1 and 26SP serum concentrations of healthy dogs and dogs with chronic diseases were measured and set in relationship to their body condition score (BCS).

2. Materials and methods

2.1. Animals

Twenty-one healthy dogs and 20 dogs with chronic diseases were included in this prospective study. Dogs which were still growing were excluded, because it is known that IGF-1 concentrations are age-dependent with higher levels during growth (Hall and Sara, 1984). The dogs were presented between 2013 and 2016 to the Small Animal Clinic of the University of Goettingen for routine health examination or diagnostic. All 41 dogs received a detailed clinical examination including assessment of the BCS (1–9) according to the WSAVA Global Nutrition Committee (1–3 under ideal/4–5 ideal/6–9 over ideal), the body weight and the shoulder height. We took blood samples from all dogs as part of a routine health examination or as part of diagnostic procedures in dogs with chronic diseases for a complete blood cell count (CBC) and serum chemistry. In addition, serum samples were analysed for IGF-1 and 26SP via ELISA. All procedures complied with regulations of the German Animal Protection Law and were carried out under the supervision of the Animal Welfare Officer, Faculty of Agriculture, University of Goettingen. As this was a clinical study, the energy intake of the dogs could not be measured, as the dogs were feed at home by their owners. The dogs received different kinds of commercial dog foods, including some special diets for their diseases, and the consumed amount of feed was not being determined.

2.2. Samples

Blood samples were collected from the cephalic vein. For CBC, the blood samples were collected in polypropylene tubes with 1.6 mg EDTA/ml blood (Fa. Sarstedt AG & Co, Nümbrecht, Germany) and were measured using a CellDyn 3500 Analyzer (Fa. Abbott GmbH & Co KG, Wiesbaden, Germany). Serum samples required for serum chemistry were collected in standard serum tubes (Fa. Sarstedt AG & Co, Nümbrecht, Germany) and were centrifuged in an Eppendorf centrifuge 5424 (Fa. Eppendorf AG, Hamburg, Germany) at 3200 $\times g$ for six minutes. Serum was analysed according to standardized procedures with a clinical chemistry analyzer (Konelab 20 i; Fa. Thermo Fischer Scientific Inc., Dreieich, Germany) and commercial kits.

For the measurement of IGF-1 and 26SP concentrations, serum samples were used. Following the commercial ELISA kit protocols (IGF-1: Cloud-Clone Corp., Houston, TX, USA; 26SP: BlueGene Biotech, Shanghai, China), samples were collected in a serum separator tube (Fa. Sarstedt AG & Co, Nümbrecht, Germany) and were kept at room temperature for 2 h to allow clotting, followed by centrifugation for 20 min (IGF-1) and 15 min (26SP) at 1000 $\times g$. Serum was extracted from the tube and was stored in aliquots at −80 °C until required for analysis. Repeated freeze-thaw cycles and a storage time of more than two months for IGF-1 or six months for 26SP were avoided.

2.3. IGF-1 assay

For measuring serum IGF-1 levels, we used the canine ELISA kit for IGF-1 from Cloud-Clone Corp. (Houston, Tx, USA), following the manufacturer's instructions. All samples and standards were measured in triplicate. The optical density (O. D.) of each well was analysed by a TECAN microplate reader (Fa. TECAN Austria GmbH, Groeding, Austria) at a wavelength of 450 nm. We used CurveExpert 1.4 software (CurveExpert, Daniel G. Hyams, Hixson, TN; https://www.curveexpert.net/) to generate the standard curves and calculate the IGF-1 concentrations (ng/ml) of the samples. The limit of detection of IGF-1 in this ELISA kit is typically < 2.42 ng/ml. The manufacturer indicates the mean intra- and inter-assay coefficients of variation with < 10% and < 12%, respectively.

2.4. 26S proteasome assay

Serum 26SP concentrations were detected using a commercially available canine 26S proteasome quantitative ELISA kit (BlueGene Biotech, Shanghai, China) following the manufacturer's instructions. All standards and samples were applied in triplicate onto the ELISA plate. The optical density (O. D.) of each well was analysed at a wavelength of 450 nm. As for IGF-1, a TECAN microplate reader and the CurveExpert 1.4 software were used to calculate the serum 26S levels. According to the manufacturer's instructions the limit of detection of 26SP in this ELISA is 0.1 ng/ml. The manufacturer indicates the mean intra- and inter-assay coefficients of variation with < 10%.

2.5. Statistical analysis

Statistical analysis was conducted using the program STATISTICA (version 12.7; StatSoft (Europe) GmbH, Hamburg, Germany). For descriptive statistics, median, minimum and maximum or range were calculated. The statistical distributions of the variables were tested graphically by evaluating histograms and quantile-quantile plots. Spearman's rank correlation was used to assess the relationship between IGF-1, 26SP or IGF-1/26SP and the BCS, as well as between IGF-1 and 26SP. In addition, the correlation of IGF-1 and 26SP with animal body weight, shoulder height and age were tested via the Spearman's rank correlation. For comparison of two groups, we used the non-parametric Mann-Whitney *U* test. A *P*-value below 0.05 was considered statistically significant.

3. Results

3.1. Results of healthy dogs

The group of healthy dogs had a median age of four years (range: 1 to 11 yr). The group consisted of 17 female dogs (seven intact and 10 neutered) and four male dogs (one intact and three neutered). Eleven dogs were of mixed breed, ten dogs pertained to different breeds. The median shoulder height was 49 cm (range: 32 to 59 cm) and the median weight was 19 kg (range: 8.2 to 29 kg). Five dogs had a BCS of four, 10 dogs showed a BCS of five and in six dogs the BCS was six.

3.1.1. Results IGF-1

In the group of 21 healthy dogs, IGF-1 concentrations were not